valent attitudes of the youth towards the most important aims of life and the demands of the environment: again, a lack of balance within the personality and the environment. It is for that reason that disturbed and retarded pubescence gives rise to a number of neuroses in the post-pubertal period, especially between the ages of 20 and 30. I have dwelt on this on a previous occasion.

Conclusions

What bearing has this clinical approach on psychotherapy? The change is just as far-reaching as was that from the suggestive to the analytical method of treatment. At that time it was discovered that the suggestive removal of the neurotic phenomenon was merely a cure on the surface, because hidden behind the neurotic symptoms were emotionally toned experiences which had to be tackled therapeutically. The physician turned from the symptom to the experience. To-day we turn from experience to the personality. Without underrating the part played by certain powerful individual experiences, and, above all, the therapeutic value of an undogmatic experiential analysis, I and my collaborators have come more and more to regard individual experiences, both infantile and current, as only providing characteristic evidence, as though they were milestones marking the personality's path of development.

To-day we have come to recognize as essential the total structure of the personality and its relation to its surroundings *in toto*. Psychogenic tangles occur in the first place when the personality becomes disunited within itself or in relation to its environment—in other words, when the constitution and the personality schema, or the personality and the demands of the outside world, are in conflict. Thus the complicated psychological phenomena of neurosis are ultimately referred back to the clear clinical basis of our established fundamental concepts of natural science, to instinct and constitutional radical, to the problem of the struggle for life and adjustment to the environment.

The function of the physician in dealing with a patient suffering from a psychogenic disorder is therefore to study carefully his present-day constitutional make-up and personality structure in relation to all the significant conditions of his milieu, and to bring them to the consciousness of the patient sympathetically and truthfully. From the existing material, however rich or poor it may be, an in itself intelligible and genuine picture of the personality will have to be constructed and brought into a tolerable relation with the environment, which itself may require to be ameliorated by active interference on the part of the physician. Thus the biological adjustment—in other words, a new and tenable attitude to the struggle of life—is brought into being.

This biological approach can equally legitimately be made to apply to the psychic field. Only thus does the personality in itself acquire ethical value, and only through the elimination of sterile inner friction does it attain its highest efficiency in the service of the community.

The fifth Congress of the Societas Oto-rhino-laryngologica will be held at Bucarest under the presidency of Professor Metzianu on September 16, when papers will be read on indications for operation in acute and chronic mastoiditis, oto-rhino-laryngology in the medicine of to-morrow, a new method of radical operation in otitis media, and surgical diathermy in oto-rhino-laryngology. The subscription is 100 francs. Further information can be obtained from the general secretary, Dr. Chavanne, 5. Place des Cordeliers, Lyons.

THE CHEMICAL DIAGNOSIS OF EARLY PREGNANCY

A METHOD BASED UPON THE DETECTION OF OESTRIOL IN THE URINE

BY

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In a recent article in this *Journal* Crew (1936), when discussing laboratory tests for the early diagnosis of pregnancy, briefly indicated the need for a chemical test, and in mentioning some of the attempts that have been made to devise such a test commented upon their failure to provide trustworthy results. Two types of chemical reaction were specifically cited: (1) the histidine reaction, first introduced as a possible means of pregnancy diagnosis by Voge (1929); and (2) the Visscher and Bowman reaction (1933–4), the basis of which is somewhat obscure. The method of Schmulovitz and Wylie (1935) falls into a slightly different category from the above tests, because it is alleged to be based upon the chemical detection of the oestrogenic hormones which are known to be excreted in greatly increased amounts in pregnancy. It requires a great deal more urine than the other tests and a more complicated technique, but as it appeared to be developed from a sounder basis it was given a short trial in this laboratory. The method was abandoned, however, when it was found that the colours produced were not specific for the oestrin compounds, and, indeed, were far more intense than could possibly result from the hormone content of the extracts from which the colours were developed.

The New Method of Diagnosis

The method about to be described makes use of a similar basis to that which was theoretically adopted by Schmulovitz and Wylie. It is concerned, however, only with the oestriol content of the urine, the oestriol having been shown by Cohen and Marrian (1934) to be the predominating oestrogenic hormone in pregnancy urine, and present to the extent of five to ten times the amount of the associated oestrone. It utilizes, moreover, a more specific reaction for this hormone—namely, the Kober phenolsulphonic colour reaction, which has been so successfully developed by Marrian and his colleagues (1936) for the purpose of the rapid estimation of the oestrin compounds in late pregnancy urine, and also as a guide to their isolation of oestriol glycuronide from the same urine.

Having had considerable experience in the use of this reaction upon extracts from late pregnancy urines, I have attempted to adapt it to early pregnancy, where the amount of available oestriol was of course very much less and the proportion of interfering substances correspondingly greater. As Marrian has shown, the oestriol of pregnancy urine is present in the form of a compound with glycuronic acid, and before the active hormone can be isolated the conjugated form has to be broken down by drastic acid hydrolysis. This preliminary hydrolysis has the effect of markedly increasing the pigmentation of the urine, and certain dark products cannot be wholly eliminated in the course of working up the extracts for the oestriol reaction. In the final stage they give a darkish-brown coloration to the Kober reagent, which in early pregnancy urines completely masks the character-

istic pale red due to the presence of minute quantities of oestriol. Failure to effect sufficient purification of the extracts of these acid-hydrolysed urines by any simple means led to the examination of other methods of accomplishing the breakdown of the glycuronide that would not at the same time introduce such complications as those referred to. Marrian in his work on the hydrolysis of the oestrin compounds showed that the release of the active hormone could also be accomplished by incubation of the urine for fourteen days, thus producing a high degree of putrefaction. As only a relatively slight increase in pigmentation accompanied this putrefactive process it was decided to find out whether the breakdown could be brought about rapidly by utilizing heavy inoculation of the urine with *B. coli* and limiting the incubation period to sixteen hours (or overnight). At first urines containing a great deal of oestrin were examined—namely, those from the later stages of pregnancy—and it was found that the amount of oestriol as ascertained by colorimetric means in extracts from such urine treated bacteriologically was fairly comparable with that in parallel extracts obtained after initial treatment by the Marrian technique of acid hydrolysis.

Two samples were then compared in strictly quantitative manner with regard to the oestriol fraction only, adopting the method of Cohen and Marrian for application to both acid-hydrolysed and bacteriologically treated portions. The results were as follows:

Sample	Acid-hydrolysed	Bacteriologically Treated
	mg. oestriol per 100 c.cm.	mg. oestriol per 100 c.cm.
I	0.77	0.59
II	0.58	0.47

Though these results showed the bacterial hydrolysis to be rather less efficient than the acid hydrolysis, the final extracts from which the colour reaction was developed were entirely colourless in the case of the former procedure, whereas in the other they were a slight brownish yellow, such pigment interfering with the colour tone of the pure oestriol reaction. From the point of view of colorimetric estimation the extracts from the urine treated by bacteria were therefore much more satisfactory. It was obvious that the amount of splitting by the bacterial hydrolysis was sufficient to warrant its trial in the detection of oestriol in the urine in the earliest stages of pregnancy.

From the beginning the results were distinctly encouraging, even on such relatively small quantities as 50 c.cm. of urine, and only occasionally was difficulty encountered in the preparation of satisfactory extracts. Originally the urine after incubation was acidified to Congo-red, then treated with 20 grammes of ammonium sulphate, preparatory to the ether extraction of the hormone. Later it was found that the final extracts were uniformly better if both acidification and ammonium sulphate were dispensed with and direct extraction was made with ether. Even then, however, there was occasionally a trace of contaminating pigment capable of yielding a brownish yellow in the oestriol colour reaction, and this was eventually eliminated by a preliminary treatment of the urine with sodium bisulphite, which reduced the original pigment of the urine by about 50 per cent. and led ultimately to the production of a final extract, which contained practically no interfering substance in the last stage of the test. The detailed technique as finally adopted is described below.

Technique

Urine Sample.—The requirement of the test is a sample of at least 50 c.cm. of early morning concentrated urine, collected into a vessel chemically clean in the sense of being entirely free from all substances which might be inhibitory to bacterial action. It is also advisable that the patient should not have had any drugs for a few days before the collection, for these often increase the pigmentation of the urine, and may also adversely affect the preliminary bacterial hydrolysis.

Preliminary Treatment.—The urine is tested for high acidity by treating a few drops with methyl-red, and if acid to this indicator is adjusted with addition of alkali until it no longer shows a pink colour. A 50 c.cm. sample is then heavily inoculated with *B. coli* and incubated overnight. To the urine after incubation, whilst it is still warm, is added approximately 0.5 gramme of sodium bisulphite. The specimen is shaken until the solid has completely dissolved and then allowed to stand for fifteen minutes.

Extraction.—The partially decolorized urine is transferred to a separatory funnel of about 150 c.cm. capacity and extracted with two lots of 40 c.cm. of ether. Occasionally some emulsification occurs, but this may be rapidly resolved by centrifugation. The combined ether extracts are washed with a little water, and then well shaken with a 30 c.cm. portion of 10 per cent. sodium carbonate. The alkali layer having been discarded, this washing process is repeated until the carbonate layer is completely colourless. The ethereal solution is then again washed with distilled water, and when the water layer has been drawn off the ether layer is further extracted with two lots of 40 c.cm. of N/10 sodium hydroxide. After separation and rejection of the upper ethereal solution the combined alkali portions containing the oestriol are treated with 25 per cent. sulphuric acid drop by drop until acid to Congo-red paper. This acidified aqueous solution is then extracted with two portions of 40 c.cm. of pure "analytical" ether, after which the ether layers are combined and washed with a little water. The ether extract is given another washing with 10 per cent. sodium carbonate, and after the rejection of the alkali the remaining ether is freed from all trace of the alkaline carbonate by two more washings with distilled water. The final clear, colourless ether solution is next transferred in two portions to a 50 c.cm. transparent silica flask and the solvent completely evaporated by immersing the flask in a large beaker of water previously heated to 70° C.; the last traces of moisture are then removed *in vacuo* by direct application of suction to the flask. The dry residue is now ready for the development of the colour reaction, using the phenolsulphonic reagent.

The Reagent.—The reagent used is that recommended by Cohen and Marrian, and consists of 3.6 parts of pure phenol with 5.6 parts of pure concentrated sulphuric acid. Small quantities only are prepared at a time; 9 c.cm. of phenol, liquified by heating, to which are added 14 c.cm. of sulphuric acid with sufficient external cooling to the container to prevent undue rise of temperature, makes a convenient amount. It can then be stored in a 25 c.cm. all-glass burette, fitted with a ground-glass stopper so as to exclude moisture from the reagent. Considerable care must be taken with the reagent, which is very hygroscopic and becomes inactive when it takes up water. In measuring out, therefore, it is important first to discard a volume equal to that portion in the tip of the burette below the stopcock, so that only completely protected reagent is used

for the reaction. Moreover, it is advisable only to use a reagent which is less than one week old.

Colour Reaction.—To the flask containing the dry residue 1 c.cm. of reagent is added from the burette, and the flask then immersed in a large water-bath previously heated to about 70° C. By frequently rotating the flask the whole of the residue distributed round the sides is brought into the reagent, and the temperature of the bath is rapidly raised to boiling point, and kept there for a period of exactly ten minutes. During the boiling period it is again advisable from time to time to rotate the reagent round the sides of the flask. The reagent at this stage has assumed a yellowish colour, and it is now cooled by holding the flask under a stream of tap-water. While this cooling process is going on 1 c.cm. of 5 per cent. sulphuric acid is slowly added, the contents of the flask being kept moving in order to bring the somewhat syrupy reagent into a homogeneous solution with the dilute acid. The product, which is still yellowish, is then reheated in the boiling-water bath for a period of two and a half minutes. A positive reaction is obtained when the original colour gradually changes over to pink or red; a negative when this change is entirely absent.

Results

Altogether sixty-five cases have been tested by this method, not including any of those which were examined in the course of the preliminary work leading up to the adoption of the above technique. In all cases the urines were those submitted for pregnancy diagnosis, so that in no instance was pregnancy established beforehand by clinical examination. The first sixty-three cases have been divided into three groups, and the results are recorded in relation to the time elapsing since the first missed period, where that information is available.

GROUP I.—*Twenty-two Cases in which Pregnancy was Established by the Friedman Reaction, and in which Clinical Details were Furnished with the Specimen, so that the Duration of the Pregnancy could be Determined*

(a) Period 2 weeks overdue .. .. 6 cases
Positive .. .. 5 „ (all reactions were weak)
Doubtfully positive .. .. 1 case

(b) Period 3 weeks overdue .. .. 6 cases
Positive .. .. 5 „
Weakly positive.. .. .. 1 case

(c) Period 4 weeks or more overdue 10 cases
Positive .. .. 10 (all fairly strong)

GROUP II.—*Eleven Cases in which Pregnancy was Excluded by a Negative Friedman Reaction, and in which Similar Clinical Information was Supplied to that given in Group I*

(a) Period 2 weeks overdue .. .. 1 case
Negative .. .. .. 1 „

(b) Period 3 weeks overdue .. .. 3 cases
Negative .. .. .. 3 „

(c) Period 4 weeks or more overdue .. 7 cases
Negative .. .. .. 7 „

GROUP III.—*Thirty Cases Submitted with no Clinical Details*

(a) Pregnancy established by the Friedman reaction .. 18 cases
Oestriol reaction { Positive, 16 / Doubtfully positive, 1 / Negative, 1 }

(b) Pregnancy excluded by a negative Friedman reaction, 12 cases
Oestriol reaction .. .. Negative, 12 cases

Since completing the above series two cases became available which gave positive Friedman reactions at a stage when the period was only one week overdue. These were submitted to the chemical test, and one gave a weak but easily recognizable reaction, while the other gave a much stronger positive, comparable to that usually obtained in Group I (*b*).

In the whole series there is only one error arising from the application of the chemical test to early pregnancy diagnosis, so that its accuracy can be said to approach that of the Aschheim-Zondek and Friedman tests. Two cases of doubtful positivity, however, would have required repetition one week later, while, on the other hand, one Friedman test had to be repeated because it gave a very doubtful reaction when the colour test showed a definite positive. The second biological test on a further sample obtained five days later confirmed the accuracy of the chemical diagnosis.

Most of the other tests that have been suggested as a means of pregnancy diagnosis have had the merit of simplicity without that of reliability. With this test the reverse holds, and it leaves something to be desired by way of simplification. However, investigation has shown that none of the steps can be sacrificed without impairing the general efficiency of the process; the only saving that might reasonably be resorted to would be to cut down to a single extraction, using about twice the volume of the extracting medium, those processes where two extractions have been adopted as the standard practice. If consideration, therefore, be made only of the time and labour involved in a single test, then it becomes doubtful whether such technique could find general application to pregnancy diagnosis. However, where larger numbers are concerned and measures are taken to carry out five or six tests simultaneously, the method warrants more serious consideration as a practical alternative to the biological methods. To carry out such simultaneous extractions all that is necessary is the construction of a mechanical shaker to hold a number of separatory funnels.

Advantages and Disadvantages of the Method

At this stage one may conveniently summarize the advantages and disadvantages of the chemical method as compared with the biological tests. In favour of the former it can be pointed out that it enables a result to be obtained in twenty-four hours, whereas the most rapid of the biological tests usually takes forty-eight hours. It dispenses with animals, the upkeep of which is expensive and which are subject to marked individual variation in sensitivity to hormone injection. Moreover, it can make use of specimens that have undergone some degree of putrefaction and have become toxic to test animals.

Its disadvantages are: first, the more laborious technique, and, secondly, that the reactions are rather weak at the stage when the period is only two weeks overdue. There is also the possibility that the chemical test may be, in general, a few days behind the Friedman test in providing positive results in the very earliest stages of pregnancy. This point, however, has not as yet been sufficiently investigated.

Even though, owing to its more tedious procedure, the method might not be adopted as a routine in pregnancy diagnosis, the very fact that it provides a much readier route to the approximate determination of excess of the preponderating oestrogenic hormone than that by way of biological assay would seem to assure to it (or perhaps some slight modification adapted to larger quantities of urine) a role of some practical importance in the field of endocrinology.

Summary

1. A biochemical test for the diagnosis of early pregnancy is described which is based upon bacterial splitting of oestriol glycuronide and the subsequent development of the oestriol colour reaction with phenolsulphonic acid.

2. Urines from sixty-five cases in which pregnancy diagnosis was required have been examined by the test. In all except one case the result was in agreement with that of the Friedman reaction. The advantages and disadvantages of the test as compared with the biological tests are briefly discussed.

In conclusion I have to express my warmest thanks to Messrs. Parke, Davis and Co. for a generous supply of pure crystalline oestriol (theelol), which has been used in this work as the standard control substance. My thanks are also due to Miss I. McPhee for her invaluable assistance in the carrying out of the many extractions, and to Dr. A. R. Berrie and Mr. T. Harvey of the bacteriological department, who kindly undertook the inoculations.

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ATHLETIC INJURIES OF THE KNEE-JOINT, EXCLUDING CARTILAGE INJURIES*

BY

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Of all athletic injuries those of the knee-joint are the most common. They often lead to prolonged unnecessary incapacity and absence from sport, especially if no definite programme of treatment is mapped out from the start. The athlete is usually a sane and sensible person as regards carrying out the treatment of his injuries, provided that the diagnosis, the difficulties of a quick recovery, and an active course of treatment in which his full co-operation is necessary are fully explained to him. Athletic injuries to this joint, as in all other injuries, may be due to: (*a*) direct violence, or (*b*) indirect violence.

Direct violence, such as a fall on the knee, a knock, or a kick, may cause any of the following:

1. A traumatic synovitis.

2. Contusion of the quadriceps muscles or their expansions. In these cases a synovitis of the knee may follow in a few days, either from seeping of blood serum into the joint or irritation of the capsule and synovial membrane by the products of bruising. Efforts are naturally made to prevent this synovitis, but the patient is warned of its possible occurrence.

3. A prepatellar bursitis or bruising of the infrapatellar pad of fat.

4. Fracture of the patella or head of the fibula.

Therefore in cases in which there is tenderness in relation to a bone a radiograph should be taken immediately.

There are four main types of indirect violence:

1. Flexion, usually producing a synovitis.

2. Rotation, which in a mild degree produces a synovitis, but if severe causes cartilage injuries.

3. Lateral wrenches, in which the lateral ligaments are involved.

4. Extension, in which the anterior cruciate ligament, infrapatellar fat and alar folds, as well as the posterior capsular structures, including the popliteus tendon, may be damaged.

Often the injury is due to a combination of two or more of the above.

Before considering in detail each variety of injury let me here stress the importance of giving the patient special instructions as to graduated remedial exercises and simple remedies which he can carry out himself. The patient is warned that at all costs he must avoid undue wasting of the quadriceps muscles and a stiff knee. A scale of graduated exercises starting with those avoiding weight-bearing includes:

1. Twitching and contracting the quadriceps muscles for two minutes every hour while lying on a couch.

2. With the legs dangling over the edge of a couch so that the knees are bent, alternately extending and flexing each knee. This exercise is carried out twice daily, starting with twenty complete movements, increasing by five movements each time.

3. Raising the extended leg by flexing the hip while lying flat; abducting the thigh at the hip and then adducting it. This exercise is designed to bring into action every muscle of the thigh and buttock.

4. Bicycling.

With each exercise the patient should be made to relax completely before starting the movement again, as it is the active contraction of the muscle from its completely relaxed state which is so important. As the patient improves and synovitis disappears graduated weight-bearing exercises can be started—such as:

5. Holding the back of a chair; heel raising and knee bending and straightening.

6. Skipping.

Simple home remedies include:

1. Sponging the knee alternately with hot and cold water, thirty seconds of each for five minutes.

2. Massaging the muscles above the joint with olive or castor oil.

3. The application at night of Scott's dressing, antiphlogistine, or lead and opium.

Let us now consider the various injuries in detail.

Internal Lateral Ligament Strains

The importance of having a clear outlook on these injuries cannot be emphasized too strongly, as they are one of the commonest causes of chronic knee complaints. The injury is caused by an abduction wrench such as takes place in turning while ski-ing, and is so common in Switzerland that it is vulgarly known as the "Swiss kiss." Sometimes rotation occurs in the injury, and an injury to the internal semilunar cartilage complicates the condition. This complication one might expect in mining injuries, but it is my experience that in athletic injuries it is uncommon for internal lateral ligament strains to be associated with cartilage injuries. In those cases though, in which the maximum tenderness is at the middle of the ligament, at or near the point of attachment of the internal cartilage to the deep surface of the ligament, and in which there is limitation of full extension from the start, damage to the cartilage should be considered as a likely complication of the strain.

Usually, however, there is marked tenderness at either its upper or lower attachment—the upper is more common—and external rotation is limited and painful. Sometimes a small flake of bone is separated from the attachment of the ligament to the internal femoral condyle. It is important, therefore, to radiograph all cases at once. The essential point to be sure of is whether extension is limited or not. In the early stages of an uncomplicated

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